Vacuum-assisted closure therapy increases local interleukin-8 and vascular endothelial growth factor levels in traumatic wounds

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Abstract: BACKGROUND: Clinical observations are suggesting accelerated granulation tissue formation in traumatic wounds treated with vacuum-assisted closure (VAC). Aim of this study was to determine the impact of VAC therapy versus alternative Epigard application on local inflammation and neovascularization in traumatic soft tissue wounds. METHODS: Thirty-two patients with traumatic wounds requiring temporary coverage (VAC n = 16; Epigard n = 16) were included. At each change of dressing, samples of wound fluid and serum were collected (n = 80). The cytokines interleukin (IL)-6, IL-8, vascular endothelial growth factor (VEGF), and fibroblast growth factor-2 were measured by ELISA. Wound biopsies were examined histologically for inflammatory cells and degree of neovascularization present. RESULTS: All cytokines were found to be elevated in wound fluids during both VAC and Epigard treatment, whereas serum concentrations were negligible or not detectable. In wound fluids, significantly higher IL-8 ($p < 0.001$) and VEGF ($p < 0.05$) levels were detected during VAC therapy. Furthermore, histologic examination revealed increased neovascularization ($p < 0.05$) illustrated by CD31 and von Willebrand factor immunohistochemistry in wound biopsies of VAC treatment. In addition, there was an accumulation of neutrophils as well as an augmented expression of VEGF ($p < 0.005$) in VAC wound biopsies. CONCLUSION: This study suggests that VAC therapy of traumatic wounds leads to increased local IL-8 and VEGF concentrations, which may trigger accumulation of neutrophils and angiogenesis and thus, accelerate neovascularization.

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Running head: Locally elevated IL-8 and VEGF during VAC™ therapy

Vacuum-Assisted Closure (VAC™) therapy increases local Interleukin-8 and Vascular Endothelial Growth Factor levels in traumatic wounds

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Abstract

Background: Clinical observations are suggesting accelerated granulation tissue formation in traumatic wounds treated with Vacuum-Assisted Closure (VAC™). Aim of this study was to determine the impact of VAC™ therapy versus alternative Epigard™ application on local inflammation and neovascularization in traumatic soft tissue wounds.

Methods: 32 patients with traumatic wounds requiring temporary coverage (VAC™ n=16; Epigard™ n=16) were included. At each change of dressing, samples of wound fluid and serum were collected (n=80). The cytokines interleukin (IL)-6, IL-8, Vascular Endothelial Growth Factor (VEGF) and Fibroblast Growth Factor (FGF)-2 were measured by ELISA. Wound biopsies were examined histologically for inflammatory cells and degree of neovascularization present.

Results: All cytokines were found to be elevated in wound fluids during both VAC™ and Epigard™ treatment, whereas serum concentrations were negligible or not detectable. In wound fluids, significantly higher IL-8 ($p<0.001$) and VEGF ($p<0.05$) levels were detected during VAC™ therapy. Furthermore, histological examination revealed increased neovascularisation ($p<0.05$) illustrated by CD31 and von Willebrand Factor (vWF) immunohistochemistry in wound biopsies of VAC™ treatment. In addition, there was an accumulation of neutrophils as well as an augmented expression of VEGF ($p<0.005$) in VAC™ wound biopsies.

Conclusion: This study suggests that VAC™ therapy of traumatic wounds leads to increased local IL-8 and VEGF concentrations, which may trigger accumulation of neutrophils and angiogenesis and thus, accelerate neovascularization.

Key words: Vacuum Assisted closure, Epigard, trauma, wound, cytokines

Introduction

The management of traumatic wounds remains a major challenge in surgery. A variety of methods of temporary wound closure have been described and used in the past. Among current standards is wound coverage by Epigard™ (Biovision GmbH, Ilmenau, Germany) or by Vacuum-Assisted Closure (VAC™, Kinetic Concepts, Inc., San Antonio, TX, USA) (*Figs. 1A and B*). Epigard™ is a two-layer, non-medicated wound dressing which approximates the function of human skin (*Fig. 1A*).[1] The superficial Teflon layer is permeable to air, however, prevents the penetration by bacteria and fluids from the outside. The lower layer is made from polyurethane. It is adhesive to the wound exudates and wound ground. Thus, removal of the Epigard™ produces mechanical debridement of the wound. The VAC™ therapy is a temporary protection of soft tissue defects by polyurethane foam which is sealed with a transparent adhesive drape (*Fig. 1B*). A negative topical pressure within the wound is then generated using a vacuum pump.[2] VAC™ therapy has been described to be beneficial for the wound management in a variety of clinical traumatic and non traumatic soft tissue defects.[2] This corroborates our clinical observation of improved wound healing of traumatic wounds after VAC™ therapy compared with application of alternative wound dressings e.g. Epigard™. It has been shown that VAC™ therapy, on the one hand, optimizes micro perfusion and blood flow, increases the partial oxygen pressure within the tissue and reduces bacterial colonialization.[2] The cyclical application of sub-atmospheric pressure, on the other hand, alters the cytoskeleton of the cells in the wound bed triggering a cascade of intracellular signals that increases the rate of cell division and subsequent formation of granulation tissue.[2] However, the exact mechanisms leading to this improved wound healing remain largely unknown.

During each of the three phases of wound healing, inflammation, proliferation and scar formation, local cellular and circulating humoral components play an important role.[3-7] Among the latter, interleukin (IL)-6 has been described as a pro- and anti-inflammatory

mediator.[8] It is a potent inducer of B-cell differentiation and immunoglobulin synthesis as well as acute phase protein expression by hepatocytes. In local trauma, increased IL-6 levels have been demonstrated in wound fluids compared with matched serum.[3] Interleukin(IL)-8 is a leukocyte specific chemoattractant cytokine and seems to play an important role for the recruitment of polymorphonuclear neutrophils (PMN) during the early inflammation phase of wound healing.[9] In addition, IL-8 is also involved in the activation of leukocytes during the tissue repair phases.[10] PMN have recently been shown to express vascular endothelial growth factor (VEGF), and following traumatic brain injury, an early increase in VEGF expression was described within 4 hours post-injury in the traumatized parenchyma associated with invasion of neutrophils granulocytes.[11,12] VEGF is a growth factor which promotes neovascularization through extension and growth of existing arterial and capillary networks.[13] Macrophages, in addition, infiltrating the wound after PMN, modulate the wound angiogenesis by releasing angiogenic factors such as fibroblast growth factor (FGF)-2 and VEGF.[14] FGF-2, originally called basic FGF, is involved in angiogenesis as well as in granulation tissue formation.[15] Whereas tissue hypoxia has been described as a potent stimulator for expression of VEGF proteins and their receptors by macrophages, FGF proteins are released secondary to cell damage.[9,16] The von Willebrand Factor (vWF) is synthesised by and stored in endothelial cells.[17] When released, vWF appears to mediate platelet aggregation and adhesion. CD 31 is also expressed in endothelial cells and therefore, immunhistochemistry for CD31 and vWF can be used for illustrating and counting microvessel density in biospies.[16]

As clinical observations and own previous investigations[18] are suggesting accelerated granulation tissue formation and wound healing of traumatic soft tissue defects undergoing VAC™ treatment, the aim of this study was to determine the impact of VAC™ therapy versus alternative Epigard™ application on local inflammation in the management of traumatic

wounds. Specifically to measure wound cytokine levels of IL-6, IL-8, VEGF and FGF-2 and furthermore to assess for neovascularization in the two treatment groups.

Patients and Methods

Patients and surgical management

A total of 32 patients suffering from traumatic soft tissue injuries requiring temporary wound closure after surgical debridement were prospectively included in the study (9 females, 23 males, mean age 46.3 years, range 17 – 82; *Table 1*). All patients were enrolled under consent guidelines approved by the Human Ethical Committee of the University of Zurich. Patients were all admitted to the investigator's hospital Trauma Centre presenting with either isolated soft tissue injuries, compartment syndrome with or without underlying fracture or open fractures of the upper or lower extremities (*Tables 2 and 3*). Excluded from the study were polytraumatized patients (ISS > 16), patients suffering from major head, torso, pelvic and spinal trauma as well as patients with clinical signs of systemic or local infection during follow up. They were excluded in order to avoid confounding effects by systemic inflammation frequently found in these patients. After emergency surgical management including fasciotomies, soft tissue debridement and fracture stabilization as appropriate, soft tissue defects were covered using VAC™ (n = 16) or Epigard™ (n = 16) according to the on call surgeon's choice. In these patients, primary closure of the wound could not be performed due to imminent compartment syndrome, skin defects after initial debridement or soft tissue conditions necessitating second look operations e.g. contaminated wounds and open fractures. When VAC™-therapy was used, the polyurethane foam was cut and fixed to the wound edge using skin staples or sutures. It was then sealed with a transparent adhesive tape and continuous negative topical pressure of 125 mmHg was applied using a vacuum pump. When Epigard™ was applied, the dressing was cut and fixed to the wound edge using skin staples or sutures. This was covered using sterile gauze dressing and bandage which stayed intact until the next change of dressing. Patients were then transferred to the intensive care unit or surgical ward according to their general condition. Considering sterility, pain control, possible further soft tissue debridement and/or wound closure, changes of dressing were performed

exclusively in the operating room 48 to 96 hours either after the emergency operation or after previous change of dressing. Number and time-point of changes of dressing depended on previous soft tissue conditions as judged by the operating surgeon. Similarly, time and type of definitive wound closure (delayed primary suture, split thickness skin grafting or muscular flap followed by split thickness skin grafting) was determined according to the responsible surgeon's assessment. Trauma management, emergency and follow up surgical procedures including soft tissue debridement and fracture fixation as well as wound closure by delayed primary suture and split thickness skin grafting were performed by experienced trauma surgeons from the author's unit (n=3). If a muscular flap was considered, plastic surgeons were involved who also performed the procedure.

Wound fluid and serum sampling

At each removal of VAC™ or Epigard™ during change of dressing or before definitive wound closure, one wound fluid and one serum sample per patient was collected. A total of 80 samples were obtained. Wound fluid samples were collected by squeezing the VAC™ sponge or the Epigard™ under sterile conditions. Samples were all collected into endotoxin free tubes (cryotubes, NUNC international). Serum and wound fluid samples were then centrifuged at 4000 rpm for 20 min at 4°C, aliquoted and stored at –80°C until further processing.

Measurement of cytokines in wound fluid and serum samples

The cytokines IL-6, IL-8, VEGF and FGF-2 were analyzed by specific enzyme linked immunosorbent assays (ELISA) using commercially available ELISA kits (R&D Systems, Abingdon, UK).

Histopathology and Immunohistochemistry of wound tissue biopsies

In addition to wound fluid and serum collection, at the first removal of VAC™ or Epigard™ dressing, tissue biopsies and attached foam samples from the center of the wound were collected, fixed in buffered formaldehyde 4% and embedded in paraffin blocks. From each paraffin block 2µm thick slides were cut and stained with Hematoxylin-Eosin (H&E). Due to the typical cytomorphology of PMN and macrophages no special stains were needed for their identification. In order to count neovascularisation, immunohistochemistry for VEGF, vWF and CD31 was performed on paraffin sections of formalin fixed tissues, using a Ventana Benchmark automated staining system (Ventana Medical Systems, Tucson, Arizona). For antigen retrieval, slides were heated with cell conditioner 1 (standard procedure). Endogenous biotin was blocked with the appropriate kit. Primary polyclonal rabbit antibody against VEGF (Neomarkers Lab Vision, dilution 1:100) was then applied and revealed with the Ventana-Red enhanced detection kit. Slides were counterstained with hematoxylin prior to glass coverslipping. CD31 and vWF immunhistochemistry were performed as described above. Primary polyclonal rabbit antibody against vWF (Dako A/S, Glostrup, Denmark, dilution 1:1000) and primary monoclonal mouse antibody against CD31 (Dako A/S Glostrup, Denmark, dilution 1:10) were used.

Neovascularization after VAC™ and Epigard™ treatment

VAC™ (n=5) *and Epigard™* (n=5) wound biopsies were investigated by an experienced pathologist (D.M) blinded to the type of wound dressing. The number of vessels, highlighted by CD31 and vWF immunhistochemistry, was counted per $1mm^2$. When the vessel density in a biopsy was heterogeneous, always the area with the highest vessel density was chosen. For VEGF expression the whole biopsy was investigated semiquantitativly with a 3 point scoring system: score 1 little, score 2 moderate and score 3 severe VEGF expression.

Statistical analysis

Data were analyzed for statistical significance using SigmaStat™ (SPSS Science Software GmbH, Erkrath, Germany). Treatment groups were compared statistically regarding gender distribution using the *Fisher exact* test, regarding age using the Student's *t*-test and regarding ISS using the Mann-Whitney *U* test. For analysis of cytokine levels within the VAC™ or Epigard™ treatment group, one way (time) repeated measures ANOVA was used. As this revealed no significant changes in cytokine concentrations over time, patient's means of cytokine levels during VAC™ versus Epigard™ therapy were compared and therefore, the Student's *t*-test or, if normality test failed, the Mann-Whitney *U* test was applied. Vessel density and VEGF expression during VAC™ versus Epigard™ therapy were analyzed using the Student's *t*-test and Mann-Whitney *U* test, respectively. A P level < 0.05 was considered statistically significant.

Results

Cytokines in wound fluid and serum after VAC™ and Epigard™ treatment

A total of 80 wound fluid and serum samples from 32 patients were analyzed and IL-6, IL-8, VEGF and FGF-2 concentrations were measured by ELISA. In 24 patients (VAC™ n = 11, Epigard™ n = 13) definitive wound closure was performed at the first change of dressing and one wound fluid and one serum sample per patient could be examined. Eight patients (VAC™ n = 5, Epigard™ n = 3) underwent one change of dressing before definitive wound closure and two consecutive wound fluid and serum samples were analyzed (*Tables 2 and 3*).

All cytokines were elevated in wound fluids at each removal of dressing during VAC™ therapy as well as Epigard™ treatment (*Table 4*). On the contrary, serum concentrations were negligible or not detectable during both treatments with VAC™ and Epigard™ (*Table 4*).

No statistically significant change of concentration over time could be detected in wound fluids during both VAC™ and Epigard™ therapy for any of the cytokines measured as assessed by one way repeated measures ANOVA. However, comparisons of patient's mean cytokine concentrations in wound fluids revealed statistically significant higher IL-8 ($p<0.001$; *Table 4*) and VEGF ($p<0.05$; *Table 4*) levels during VAC™ therapy compared with Epigard™ application. In contrast, a statistically significant difference between VAC™ and Epigard™ treatment was found neither for IL-6 and FGF-2 concentrations in wound fluids nor for any cytokine measured in serum.

Local accumulation of PMN in wound tissue after VAC™ therapy

Histological examination of wound ground biopsies with attached VAC™ foam showed visible higher accumulation of PMN (*Figs. 2A and B*) compared to wound ground biopsies with Epigard™ dressing (*Figs. 2E and F*). This is in accordance with the locally increased

levels of IL-8 in wound fluids during VAC™ therapy. In both treatment groups spare macrophages were identified.

Increased expression of VEGF and corresponding augmented neovascularization in wound tissue after VAC™ therapy

Wound biopsies treated with VAC™ therapy showed immunohistochemically visible higher expression of VEGF (*Fig. 2C*) compared to Epigard™ application (*Fig. 2G*), which is corroborated statistically by semiquantitative analysis ($p<0.005$). VEGF was mostly expressed in cells identified as PMN.

In addition, vWF and CD31 immune staining, which visualizes small vessels, showed a statistical significant increased neovascularisation after VAC™ (33.6 ± 2.9, mean ± SEM) therapy (*Fig. 2D*) compared to Epigard™ (14.0 ± 3.3) treatment ($p<0.05$) (*Fig. 2H*).

Clinical outcome

Clinical outcome parameters are presented in *Tables 2 and 3*. Although significantly higher cytokine levels, PMN accumulation as well as expression of VEGF and vWF could be shown during VAC™ therapy compared with Epigard™ application, clinical results were similar in both treatment groups showing no significant overall differences in closure time and hospital stay.

Discussion

Although in clinical settings improved and accelerated granulation tissue formation and wound healing has been attributed to VAC™ therapy compared to alternative wound dressings such as Epigard™, the exact underlying mechanisms remain largely unknown. Experimental studies have previously shown that mechanical stress can induce tissue responses including cytokine release and cell proliferation.[19-22] Thus, it can be hypothesized that VAC™ therapy may cause mechanically triggered immunomodulation as well as neovascularization and/or angiogenesis, finally leading to improved wound healing. In our present study, therefore, the impact of VAC™ therapy versus alternative Epigard™ application on local inflammation and neovascularization was assessed. The cytokines IL-6, IL-8, VEGF as well as FGF-2 were measured and compared in wound fluids and serum samples of 16 patients treated with VAC™ therapy and 16 patients treated with Epigard™ dressing for traumatic soft tissue injuries requiring temporary wound coverage (*Tables 2 and 3*). Furthermore, wound biopsies were processed for histological examination. The specimens were evaluated as to the presence of PMN, VEGF expression and neovascularization.

In the present study, all the cytokines measured were elevated during the whole study period in wound fluids of both patient groups treated either with VAC™ or Epigard™, whereas serum concentrations remained at constant low levels. These findings are corroborated by a previous report of markedly higher cytokine and growth factor levels in wounds compared with serum in a clinical model of controlled operative plastic surgery trauma,[3] indicating a compartmentalization of the immune response after local soft tissue injuries.

In response to traumatic injuries, IL-6 appears to play an active role in the post injury immune cascade.[23] In local soft tissue injuries e.g. elective surgery, IL-6 serum levels were increased within 90 minutes of skin incision being elevated up to 72 hours before decreasing.[23] This corroborates our presented results of low or not detectable IL-6 serum

levels measured at the first removal of dressing performed within 48 to 96 hours post-injury. In contrast to serum concentrations, however, we found IL-6 levels in wound fluids to be constantly elevated in all patients treated with VAC™ or Epigard™, indicating ongoing local inflammation independent from the type of dressing.

Similar to IL-6, FGF-2 levels did not differ in wound fluids of patients treated with VAC™ or Epigard™ and thus, indicating releasing mechanisms other than provoked by VAC™ therapy. Trengove *et al.* postulated that the highly proteolytic extracellular environment of chronic wounds may have direct effects on the levels of FGF, which is a matrix bound growth factor.[24] Interestingly, in the same study a direct relationship between IL-6 and FGF was found which could explain our findings of comparable FGF-2 levels in wound fluids during both VAC™ and Epigard™ treatment.

In contrast to IL-6 as well as FGF-2, significantly higher local IL-8 concentrations were detected in wound fluids of patients during VAC™ therapy compared with Epigard™ application. The increased partial oxygen pressure in the local wound tissue during VAC™ therapy may be responsible for these several fold higher IL-8 levels during VAC™ therapy, as macrophages and endothelial cells upregulate IL-8 under this condition.[25,26] Furthermore, increased IL-8 levels in wound fluids during VAC™ therapy accompanied by local accumulation of PMN, as demonstrated histolgically in this study, can explain clearance of local debris and contamination during VAC™ treatment,[2] finally resulting in earlier and more reliable wound closure as previously reported.[27]

In severely ischemic and/or hypoxic wound conditions, increasing oxygen concentrations result in accelerated wound healing with increased blood vessel growth.[28,29] *In vitro* studies demonstrated that hyperbaric oxygen leads to upregulation of platelet derived growth factor (PDGF) receptor in the presence of oxidants such as hydrogen peroxide, stimulating endothelial cells and keratinocytes to release VEGF.[30,31] Similarly, an experimental wound model in rats showed increased VEGF levels of approximately 40%

under hyperbaric oxygen therapy.[32] In addition, clinical and experimental studies have shown that mechanical stretch triggers VEGF secretion.[33-35] Taken together, this supports our clinical findings of significantly higher VEGF levels in wound fluids during VAC™ therapy compared with Epigard™ application. Recent studies have demonstrated PMN and neutrophils in particular to express VEGF.[11,12] Thus, the elevated local VEGF in wound fluids during VAC™ therapy in our study can be explained by increased and early accumulation of neutrophils as shown histologically. Elevated levels of VEGF were also demonstrated in our previous investigation where cytokines were measured in wound fluids of patients with traumatic wounds treated initially with Epigard™ followed by VAC™ therapy.[18] These results prompted us to assess the initial impact of VAC™ versus Epigard™ treatment on cytokine levels in wound fluids and local early inflammation. In addition to PMN, macrophages are known to be a source of VEGF peaking around the fifth day after injury in wound infiltration.[36] Interestingly, a more recent study also described an autocrine amplification mechanism for VEGF that induces chemotaxis of human neutrophils in extravascular tissue.[37] Finally, supported by our findings in wound biopsies of increased VEGF expression in PMN and presence of augmented small vessels during VAC™ treatment, it can be hypothesized that VAC™ therapy triggers neovascularization in traumatic soft tissue wounds.

The application of VAC™ as temporary wound coverage not only supports wound conditioning and facilitates definitive wound closure but also decreases frequency of dressing changes and risk of wound infection, offering considerable advantages regarding patient's comfort and hospital hygiene.[2] VAC™ therapy has been described to be beneficial for the wound management in a variety of complex soft tissue conditions such as destruction of chest wall musculature from trauma, empyema of local infection as well as infected war time missile injuries.[27,38,39] In contrast, our study could not reveal any significant difference regarding time point of definitive wound closure as well as hospital stay between patients receiving VAC™ therapy and patients treated with Epigard™. However, closure time as well

as hospital stay in particular are influenced by a variety of medical and non medical factors in these patients, and therefore, might not directly reflect advantages or disadvantages of any particular wound dressing. Thus, it can be hypothesized that the apparent positive effects of VAC™ therapy could be more accentuated in the treatment of other patients with more complex soft tissue wounds and therefore, additional clinical studies are necessary to further establish VAC™ therapy as a wound care modality.

These are the first data to demonstrate a selective and locally amplified humoral and cellular immune reaction as well as accelerated neovascularization during VAC™ therapy of traumatic soft tissue wounds compared with alternative Epigard™ application. However, further investigations are necessary in order to fully elucidate the exact mechanisms and kinetics of the wound healing process during VAC™ therapy.

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Table 1: Demographic data and Injury Severity Score (ISS) of patients with traumatic wounds treated with VAC™ and Epigard™

	VAC™	**Epigard™**
Age[1)]	45 ± 5.3	47 ± 3.6
Gender distribution[2)]	4 x female; 12 x male	5 x female; 11 x male
ISS[3)]	7.9 ± 0.8	9.6 ± 1.0

Basic demographic data and ISS of 32 patients with traumatic wound treated either with VAC™ (n = 16) or Epigard™ (n = 16) dressing. Values represent means ± SEM.

1) P > 0.05 VAC™ versus Epigard™ (Student's *t*-test)
2) P > 0.05 VAC™ versus Epigard™ (*Fisher exact* test)
3) P > 0.05 VAC™ versus Epigard™ (Mann-Whitney *U* test)

Table 2: Clinical data of patients treated with VAC™

patient n°	type of injury 1)	site	mechanism	n° of changes of dressing	closure type	closure time 2)	hospital stay (days)
1	soft tissue injury	thigh	knife stab wound	1	skin suture	4	7
2	soft tissue injury	thigh	motorbike accident	2	split thickness skin graft	5	14
3	soft tissue injury	leg	animal (boar) bite	1	skin suture	2	14
4	soft tissue injury	leg	crushed between train and train platform	1	split thickness skin graft	3	9
5	soft tissue injury	elbow	joint dislocation	1	muscular flap	11	36
6	compartment syndrome	leg	gunshot injury	2	skin suture	4	9
7	compartment syndrome	leg	sports injury (soccer)	1	skin suture	4	7
8	compartment syndrome	leg	motorbike accident	1	skin suture	3	7
9	compartment syndrome	arm	crushed by industrial machine	2	skin suture	6	10
10	compartment syndrome/closed fracture	leg/proximal tibia	pushbike hit by car	2	split thickness skin graft	25	40
11	compartment syndrome/closed fracture	midfoot/forefoot	hit by heavy plate at work	1	muscular flap	8	35
12	open fracture (II)	tibia and fibula	motorbike accident	1	muscular flap	3	37
13	open fracture (II)	olecranon	car accident	1	skin suture	2	9
14	open fracture (III)	calcaneus	pushbike accident	1	muscular flap	6	46
15	open fracture (III)	humerus	car accident	1	split thickness skin graft	2	15
16	open fracture-dislocation (III)	ankle	crushed by heavy load at work	2	muscular flap	5	16

1) Gustilo-Anderson[40] classification of open wounds
2) Days from first VAC™ application to definitive wound closure

Table 3: Clinical data of patients treated with Epigard™

patient n°	type of injury 1)	site	mechanism	n° of changes of dressing	closure type	closure time 2)	hospital stay (days)
17	soft tissue injury	leg /foot	motorbike accident	1	muscular flap	8	6
18	compartment syndrome	thigh	sports injury (skiing)	1	skin suture	3	20
19	compartment syndrome	leg	pushbike accident	1	skin suture	5	13
20	compartment syndrome	leg	pedestrian hit by car	1	skin suture	3	13
21	compartment syndrome / closed fracture	leg/proximal tibia	car accident	1	split thickness skin graft	6	25
22	compartment syndrome / closed fracture	leg/proximal tibia	fall from 2 meters	1	skin suture	6	38
23	compartment syndrome / closed fracture	leg/tibia and fibula	pedestrian hit by motorbike	1	skin suture	5	19
24	compartment syndrome / closed fracture	leg/ankle	simple fall	1	skin suture	7	16
25	open fracture (II)	tibia and fibula	gunshot injury	1	split thickness skin graft	2	19
26	open fracture (II)	calcaneus	crushed by industrial machine	1	muscular flap	8	33
27	open fracture (III)	distal femur	hit by ruptured steel cable	1	muscular flap	4	37
28	open fracture (III)	tibia and fubula	fall from 8 meters	2	split thickness skin graft	4	33
29	open fracture-dislocation (III)	ankle	car accident	2	muscular flap	15	27
30	open fracture-dislocation (III)	ankle	simple fall	1	split thickness skin graft	9	28
31	open fracture (IIIC)	distal femur	gunshot injury	2	split thickness skin graft	16	30
32	open fracture (IIIC)	proximal tibia	car accident	1	skin suture	4 (amputation)	16

1) Gustilo-Anderson[40] classification of open wounds (IIIC indicating major vascular injury)
2) Days from 1st Epigard™ application to definitive wound closure

Table 4: Cytokine levels in wound fluids and serum during VAC™ and Epigard™ therapy of patients with traumatic soft tissue injuries.

	wound fluid			serum		
	VAC™	**Epigard™**	**P value**	**VAC™**	**Epigard™**	**P value**
IL-6 [3]	36999.4 ± 15735.7	57246.8 ± 38955.5	P = 0.397	not detectable	20.1 ± 18.0	not determined
IL-8 [3]	407.9 ± 175.5	56.4 ± 11.4	P < 0.001	17.1 ± 8.6	10.0 ± 6.8	P = 0.927
VEGF [pg/ml]	8396.5± 762.7	5364.4 ± 695.0	P = 0.006	584.3 ± 105.2	572.8 ± 95.9	P = 0.936
FGF-2 [pg/ml]	361.9 ± 43.9	452.4 ± 76.9	P = 0.272	83.6 ± 48.7	65.3 ± 35.0	P = 0.983

32 Patients with traumatic wounds were treated either with VAC™ (n = 16) or Epigard™ (n =16) dressing. At each removal of VAC™ or Epigard™ during change of dressing or before definitive wound closure, one sample of wound fluid and serum per patient were collected. A total of 80 samples were collected (VAC™ n = 42; Epigard™ n = 38). Cytokines were measured by ELISA. As repeated measures ANOVA revealed no significant change of cytokine concentrations over time, values represent means of patient's means ± SEM.

Figure 1

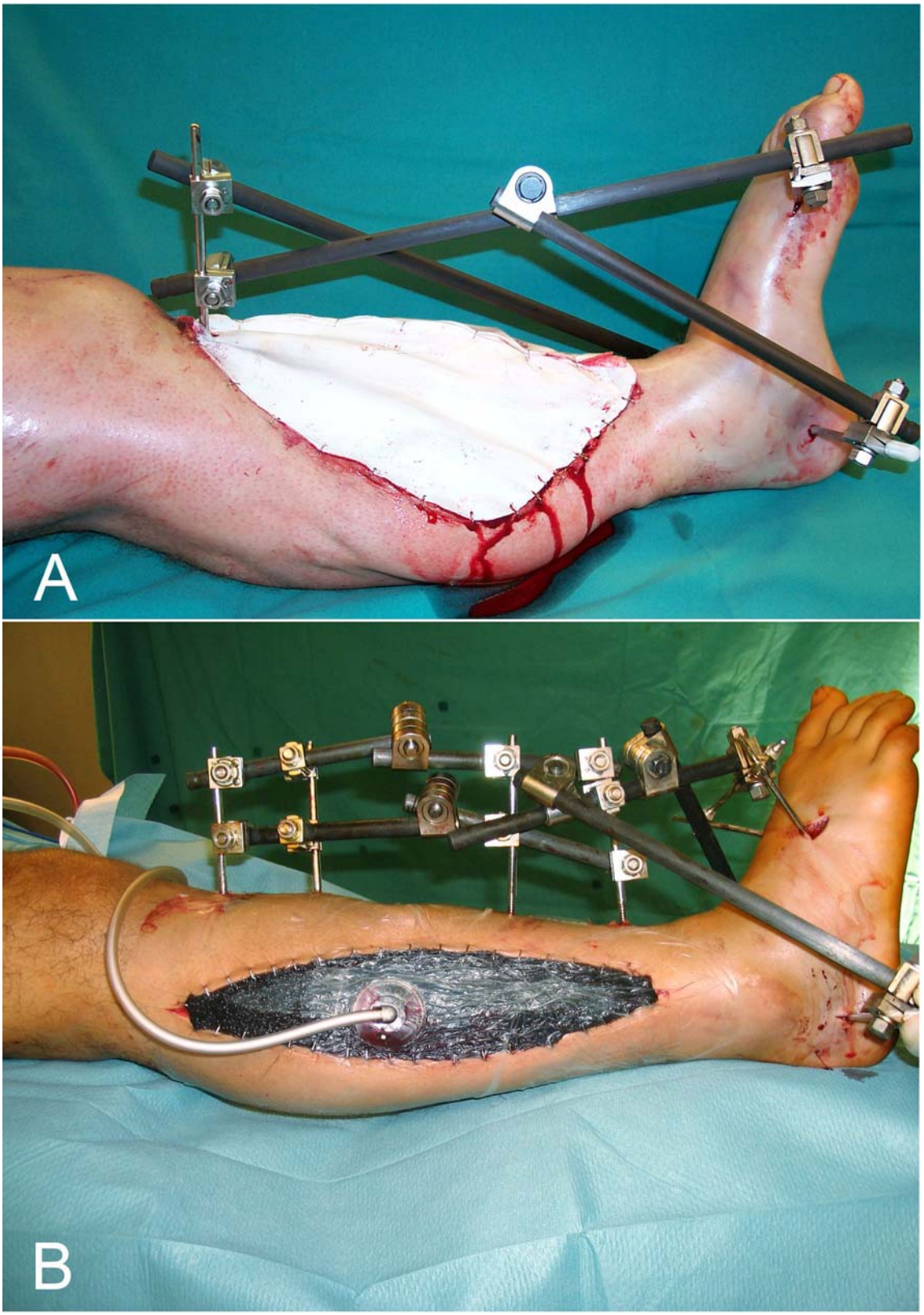

Figure 2

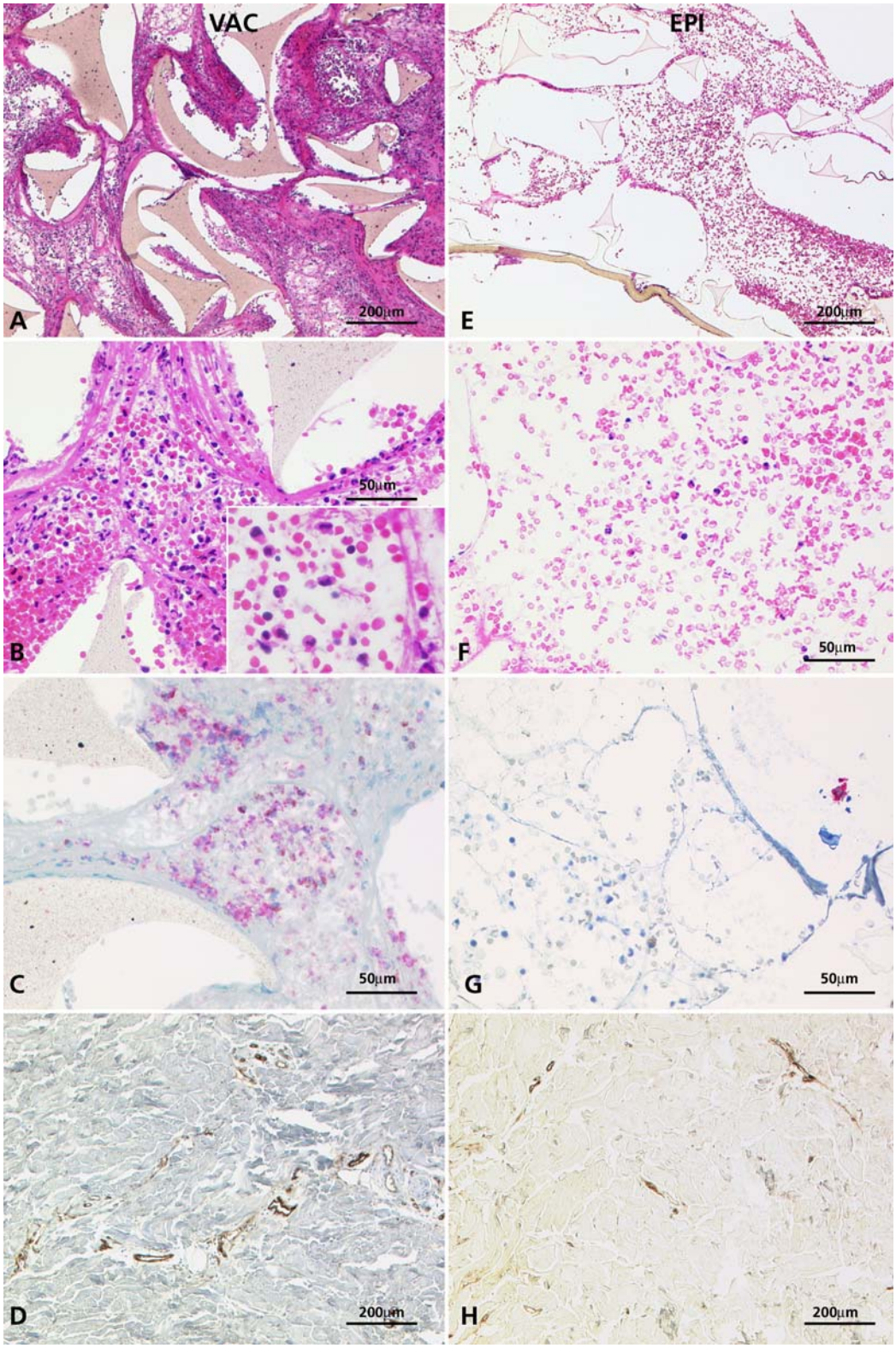
VAC
EPI
A
E
200µm
200µm
B
50µm
F
50µm
C
50µm
G
50µm
D
200µm
H
200µm

Figure Legend

Figure 1

Clinical pictures of traumatic wounds treated either with Epigard™ dressing (**A**) or Vacuum-Assisted Closure (VAC™) therapy (**B**). 32 Patients were admitted to the investigator's hospital Trauma Centre presenting with either isolated soft tissue injuries, compartment syndrome with or without underlying fracture, open fractures or traumatic amputation of the upper or lower extremities. After emergency surgical management including fasciotomies, soft tissue debridement and fracture stabilization as appropriate, soft tissue defects were covered using Epigard™ (**A**) or VAC™ (**B**).

Figure 2

Representative tissue biopsies of patients with traumatic soft tissue injuries treated either with VAC™ (**A, B, C and D**, patient number 12 in Table 2, 72 hours after initial VAC™ application) or Epigard™ (**E, F, G and H**, patient number 25 in Table 3, 48 hours after initial Epigard™ application). Biopsies with attached foam samples from the center of the wound were collected at the first removal of VAC or Epigard dressing 48 to 96 hours after the emergency operation as described in detail in *Patients and Methods*. A: Overview. VAC™ foam with accumulation of polymorphonuclear neutrophils (PMN), Hematoxylin&Eosin (H&E), x75. B (including inset) and C: Magnification of picture A. B: H&E, x300; inset: PMN, H&E, x400. C: High expression of vascular endothelial growth factor (VEGF), x300. D and H: Wound tissue with high neovascularisation in VAC™ patients (D) compared to low neovascularisation in Epigard™ patients, CD31, x75. E: Overview. Wound coverage with Epigard™ and few PMN, H&E, x75. F and G: Magnification of picture E. F: H&E, x300. G: Low expression of VEGF, x300.